

# **TRAnslational research in Clinical Oncology (TRACO)**

# Program Director

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# Organizing Committee

**Irwin Arias**

**Oliver Bogler**

**Terry Moody**

**Lyuba Vartikovski**

**Farah Zia**

# SYLLABUS

DATE	TOPIC	SPEAKERS
Sept. 7	Introduction, Cervical cancer	Moody, Schiller
Sept. 13	Global Health and Cancer, Prostate Cancer	Gopal, Madan
Sept. 20	Ovarian cancer, Immune checkpoints	Annunziata, Goff
Sept. 27	Small molecules, Clinical Trials	Simeonov, Smith,
Oct. 4	Radiation oncology, Tumor imaging	Nichols, Choyke

# SYLLABUS, continued

DATE	TOPIC	SPEAKERS
Oct. 12	COVID-19 and cancer, Cannabis and cancer	Dahut , Freedman
Oct. 18	Genomics, CAR-T cells	Wei, Brudno
Oct. 25	Breast cancer, SCLC	Zia, Chen
Nov. 1	KRAS, Brain cancer	Luo, Timmer
Nov. 8	NSCLC, HIV	Szabo, Maldarelli

# SYLLABUS, continued

DATE	TOPIC	SPEAKERS
Nov. 15	Epigenetics, Case reports	Verma, Olaku
Nov. 22	Precision medicine, Topoisomerase	Harris, Pommier
Nov. 29	Pancreatic cancer, Nanotechnology	Hussain, Dobrovolskaia

# REGISTRATION

The course is open to all interested personnel without charge. Registration is available at the NCI Web site

(<http://www.cancer.gov/grants-training/training/resources-trainees/courses-fellowships/translational-research-clinical-oncology>). The lecture PDFs will be posted on the website after they are made 508 compliant. WEBEX chats will be taken at the end of each lecture.

# Lecture recordings

- The archived lectures will be on available on WEBEX. The 2 hour lecture for Sept. 7 will be TRACO1. The number will increase each week and the Nov. 29 lecture will be TRACO13.



# **COURSE CERTIFICATION**

**Registrants can obtain a course certificate upon passing a computer graded final examination.**

# Lung, colon, breast and prostate cancer account for half of the U.S. cancer mortalities.

TYPE	INCIDENCE	(MORTALITY)
Lung	171,900	(157,200)
Colon/Rectum	147,500	(57,100)
Breast	211,300	(39,800)
Prostate	220,900	(28,900)
Others	582,500	(273,500)
Total	1,334,100	(556,500)

*Thun, Jamal and Ward, "Cancer: Principles & Practice of Oncology." Edited by DeVita, Lawrence and Rosenberg. (2011), pp. 241-260*

# **Cancers which kill 10,000-30,000 U.S. patients annually include:**

- **Pancreatic cancer**
- **Non-Hodgkin's Lymphoma**
- **Leukemia**
- **Stomach cancer**
- **Ovarian cancer**
- **Brain cancer**
- **Liver cancer**
- **Bladder cancer**
- **Esophageal cancer**
- **Kidney cancer**

# Cancer risks include:

- Alcohol
- Asbestos
- Diet
- Familial
- Hormones

# Cancer risks (continued)

- **Obesity**
- **Ion Radiation**
- **Tobacco**
- **U.V. Radiation**
- **Viral**

# **Lung Cancer kills over 150,000 patients in the U.S. annually.**

- **There are 45 Million current smokers and 45 Million ex-smokers in the U.S.**
- **It is difficult to quit smoking due to nicotine addiction.**

# **Carcinogens which have been identified in cigarette smoke include:**

- **Polyaromatic hydrocarbons (PAH),**
- **aza-arenes,**
- **4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK),**
- **1,3 butadiene,**
- **ethyl carbamate,**
- **ethylene oxide,**
- **nickel, chromium, cadmium,**
- **polonium, arsenic**
- **hydrazine**

**The process by which unreactive carcinogen converts to a form which binds DNA is known as metabolic activation.**

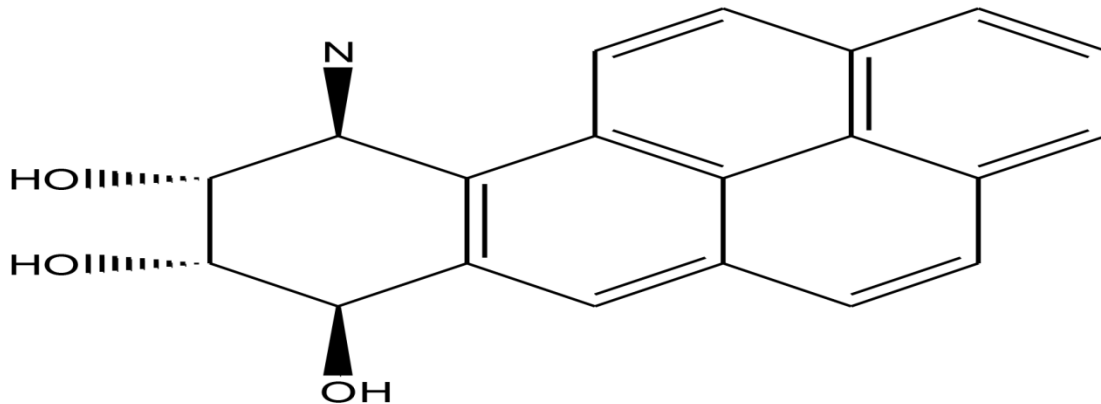
- Bay region diol epoxides are the principal PAH metabolites involved in DNA adduct formation. For Benz[a]pyrene (BaP), BaP-7,8-diol-9,10-epoxide (BPDE) forms adducts with DNA leading to G:C>T:A mutations in pulmonary DNA. The genes for p53 and k-ras are frequently mutated.**



# BENZ(a)Pyrene

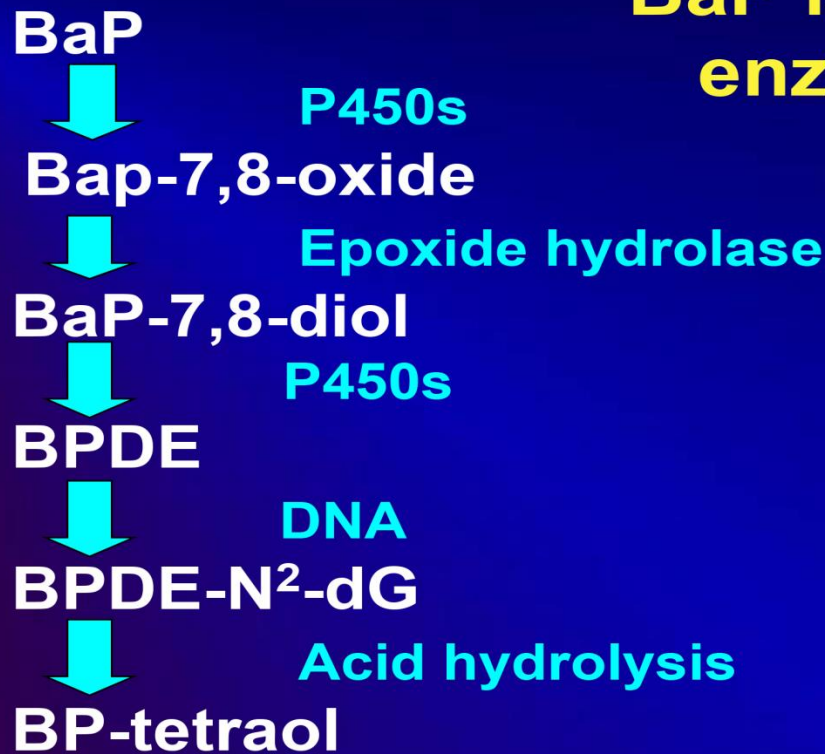
## BENZ(a)Pyrene

- The chemical structure of BaP is shown.



# BaP is metabolized to BPDE

BaP is metabolized by enzymes to BPDE.



*Boysen and Hecht, Mutation Res. 543:17(2003).*

**DNA is mutated if the rate of carcinogen activation exceeds the rate of carcinogen detoxification and/or DNA repair.**

- **DNA adducts as well as intra- and inter-strand DNA crosslinks are removed by nucleotide excision repair.**

# **Carcinogens can be detoxified and excreted prior to DNA damage.**

- **Cytochrome p450 enzymes catalyze addition of an oxygen to the carcinogen, increasing its water solubility.**
- **Phase 2 enzymes convert the oxygenated carcinogen to a form that is highly soluble in water, converting it to a form that can be excreted.**

# **P53, a tumor suppressor gene:**

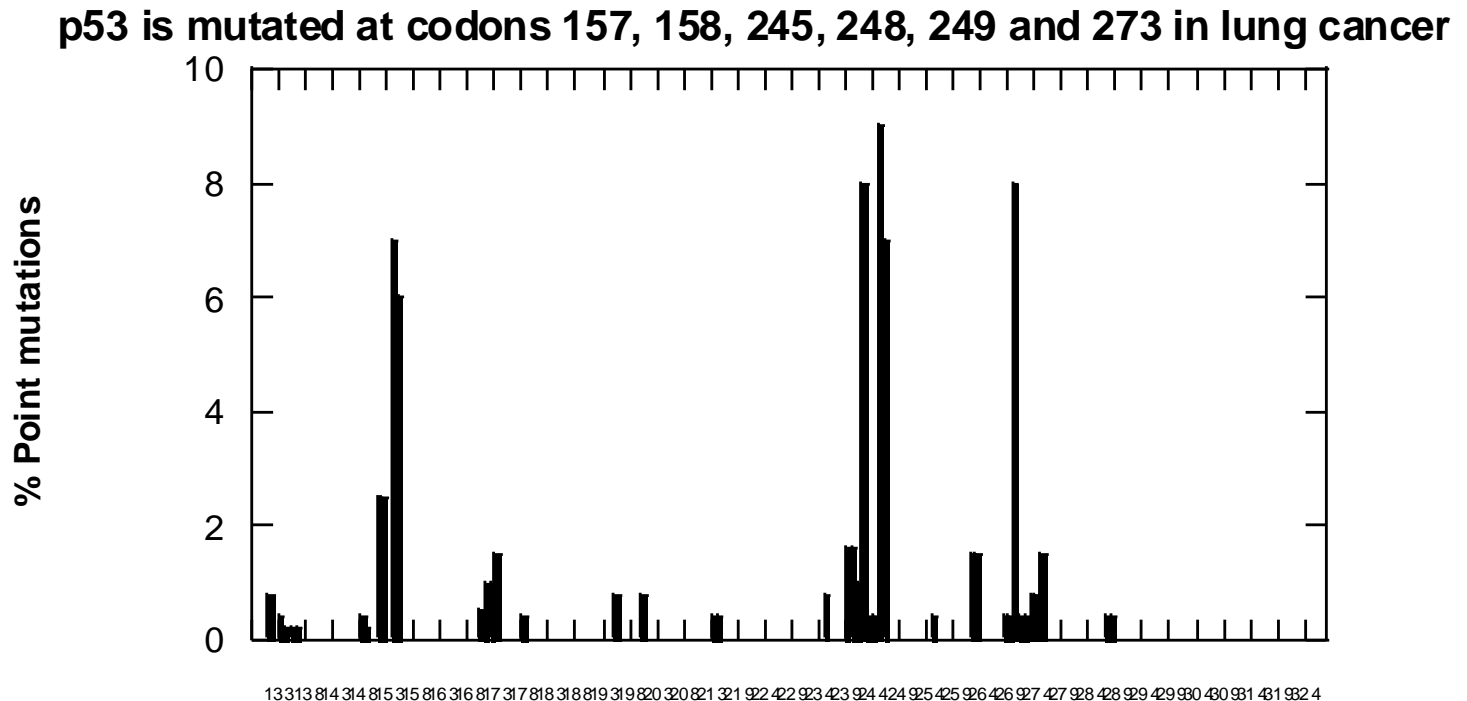
- mediates the G1 to S-phase checkpoint of the cell cycle,
- drives programmed cell death or apoptosis after DNA damage,
- is increased along with p21 (cell cycle checkpoint) after DNA damage.
- Phosphorylated p53 induces expression of BAX (apoptosis), GADD45 (DNA repair) and thrombospondin (angiogenesis)

**P53 mutations are detected in most of the lung cancer patients.**

**G to T transversions occur at the CpG rich codons including 153-158 (exon 5), 248 and 249 (exon7) and 273 (exon 8) of the p53 gene. There is an excess of G to T transversions in smokers relative to non-smokers.**

# P53 mutations.

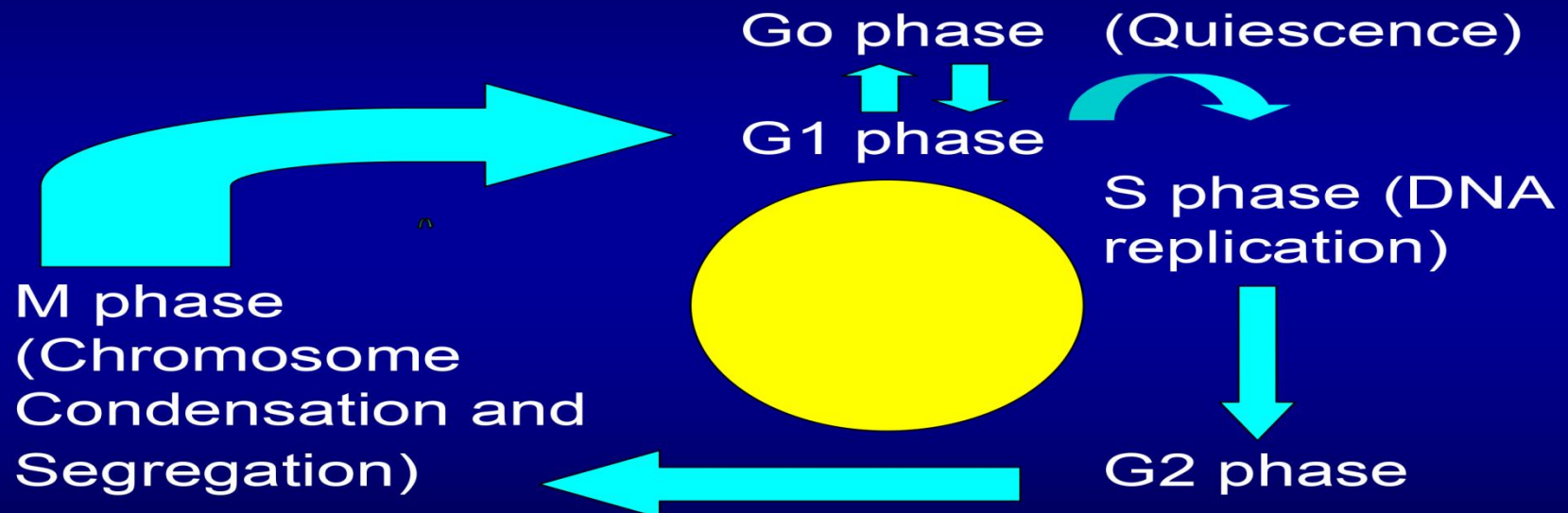
- P53 is mutated at codons 157, 158, 245, 248, 249 and 273 in lung cancer.



# Cell cycle phases

## Cell cycle phases.

- Cell cycle phases include G1, S, G2 and M





# **p53 mediates the G<sub>1</sub> to S-phase checkpoint of the cell cycle**

- **DNA damage increases p21 and p53.**
- **P53 drives programmed cell death or apoptosis after DNA damage**

# Cell cycle enzymes

## Cell cycle enzymes.

- Cyclin D/cdk is inhibited by p21,27,57,15,16,18 and 19.



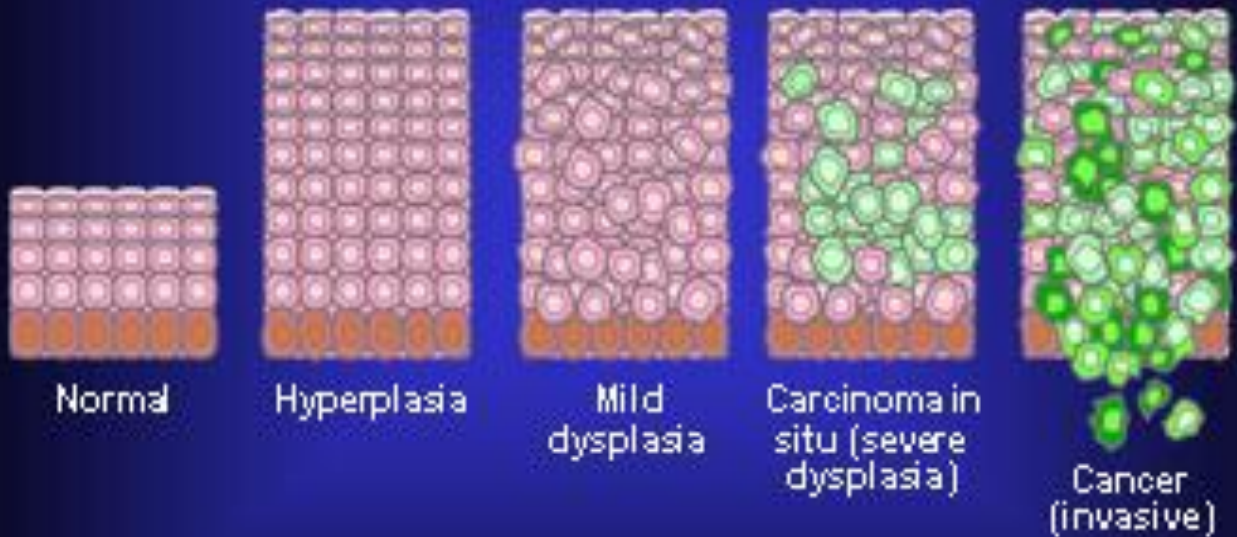
# **Genotoxicity of tobacco smoke.**

- **After 10 years of chronic cigarette smoking, normal lung tissue can undergo hyperplasia and metaplasia.**
- **After 15 years, dysplasia can result.**
- **After 20 years, a carcinoma in situ can form.**
- **After 25 years, a malignant cancer can form.**

# Carcinogenesis

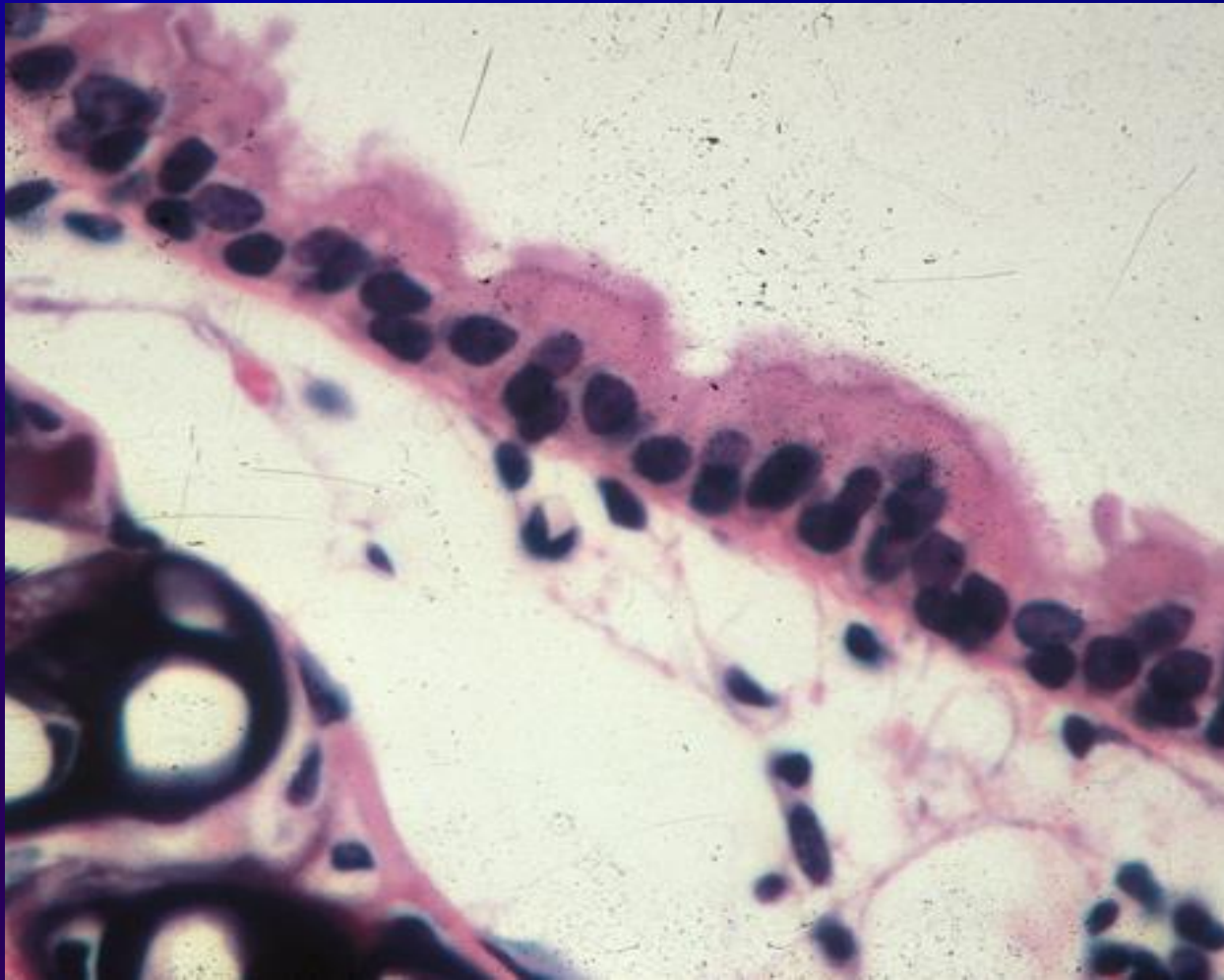
- Cancer progression occurs over a period of decades.

## Carcinoma in Situ



## Normal lung

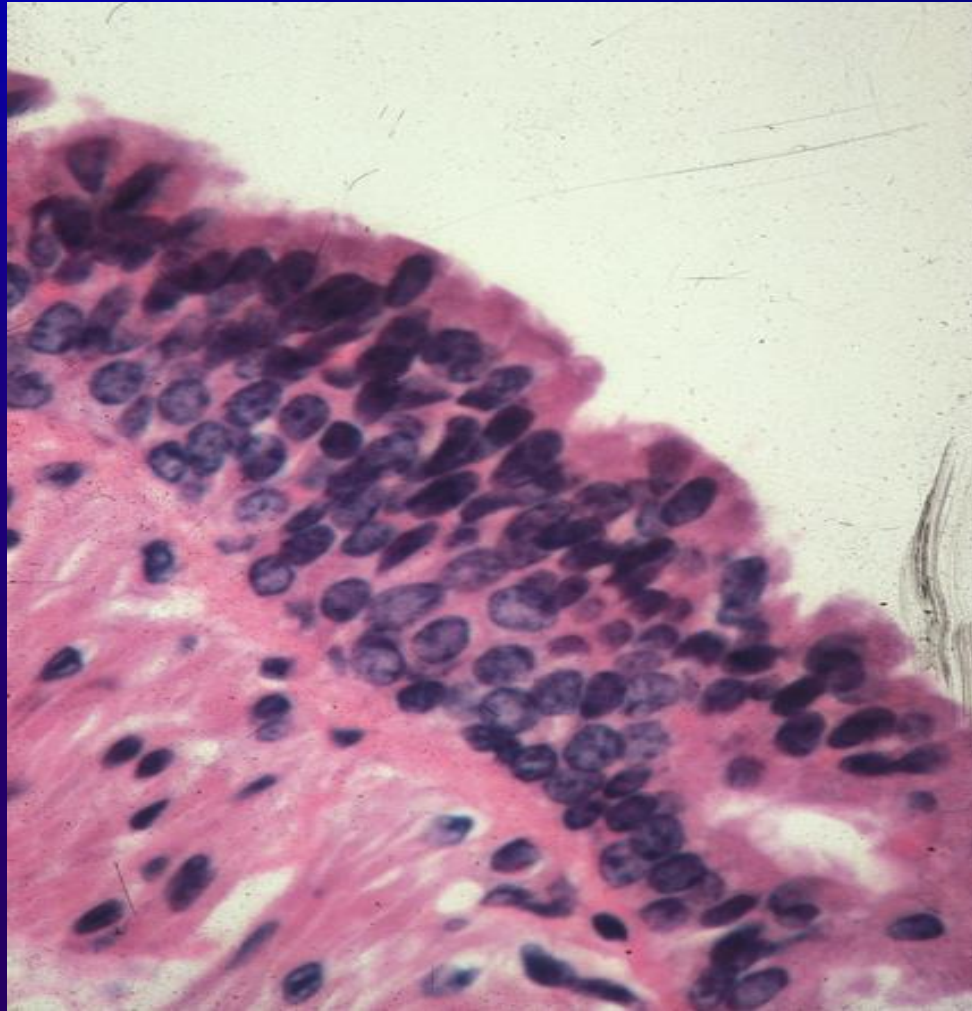
- Carbon dioxide is exhaled from the lung whereas oxygen is inhaled.





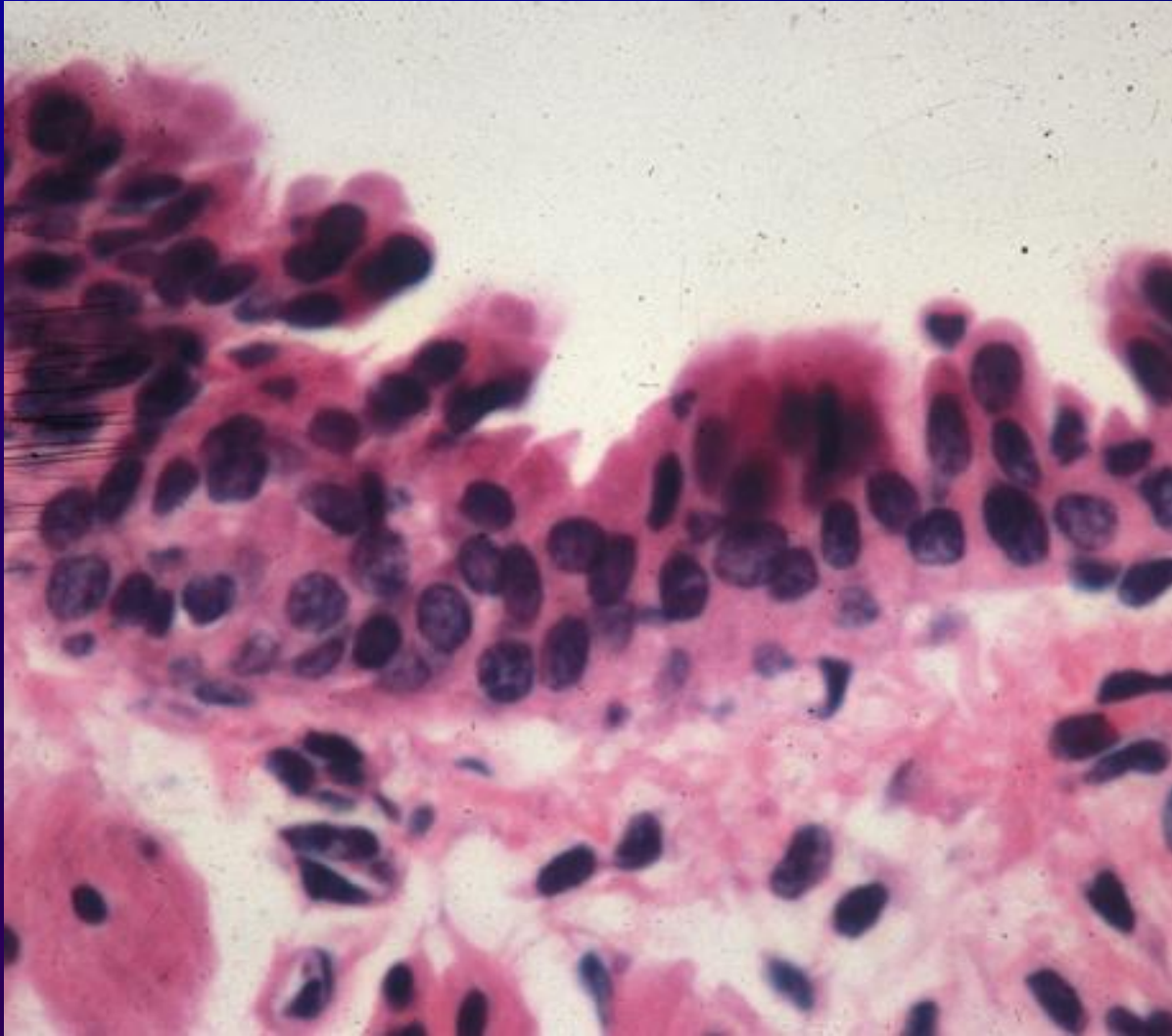
# Hyperplasia

- After exposure to tobacco smoke, hyperplasia can result.



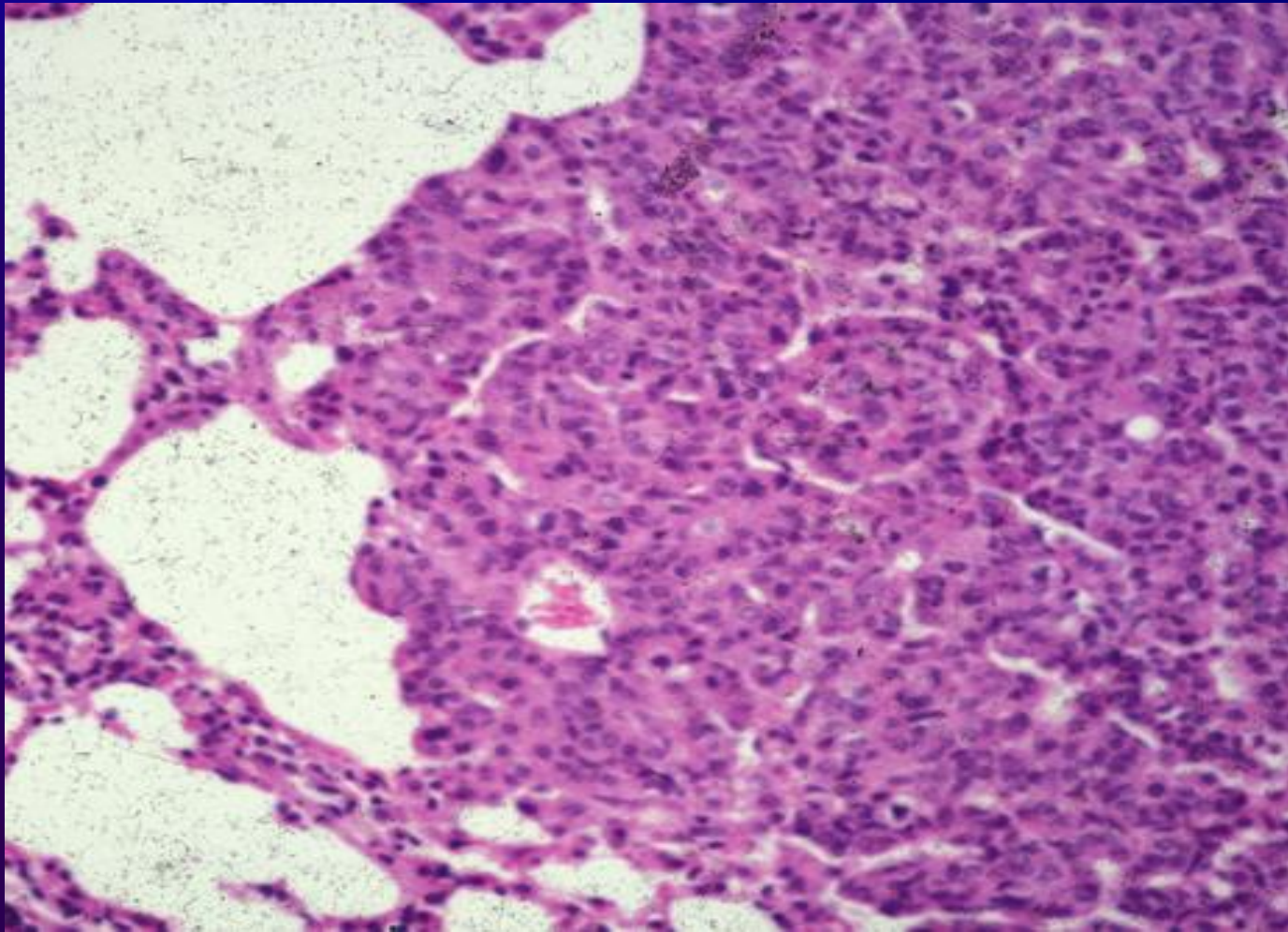
# Dysplasia

Continued exposure to tobacco smoke leads to dysplasia.



# Adenoma

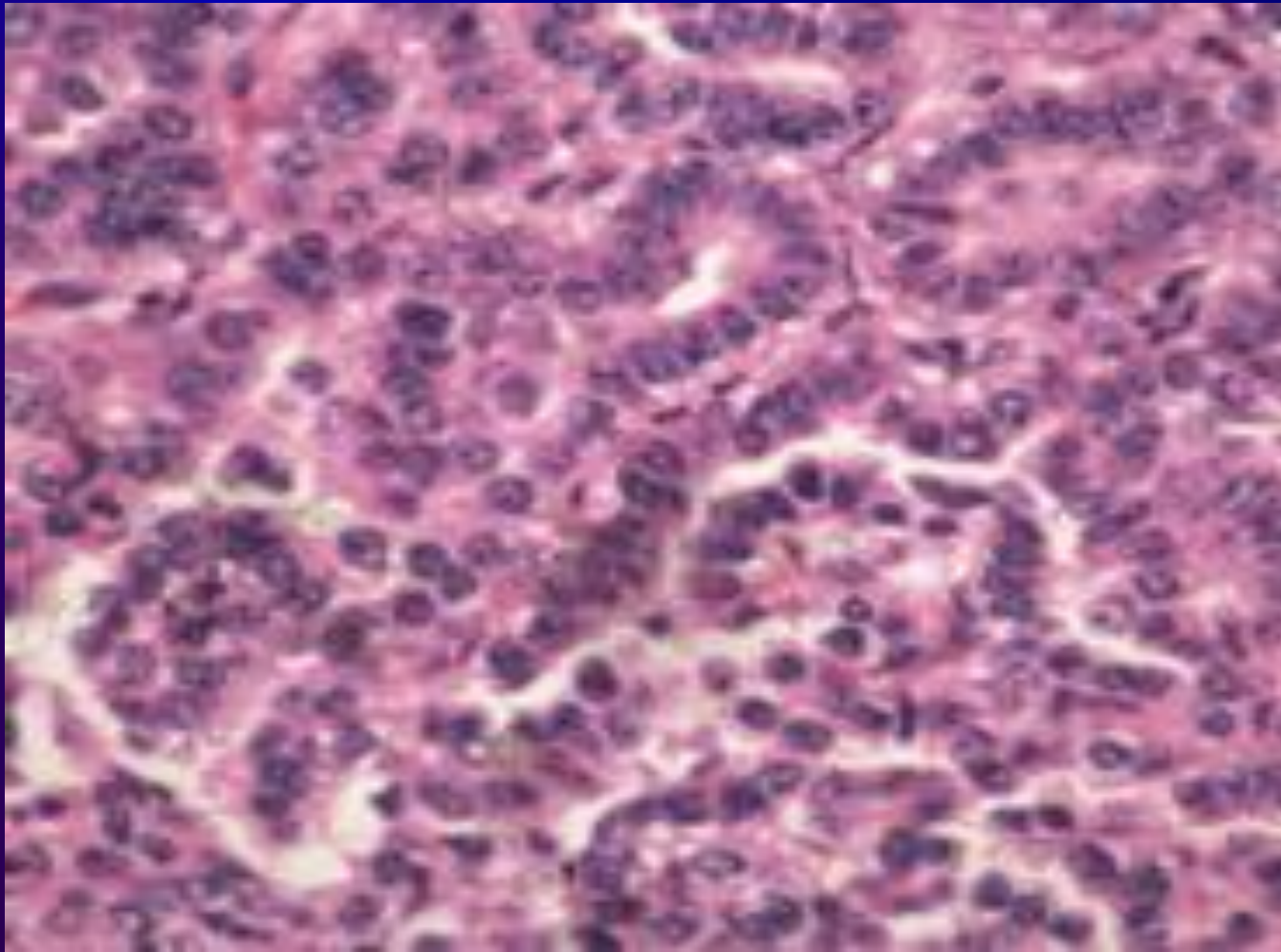
- Continued exposure to carcinogens leads to benign tumors such as adenomas.





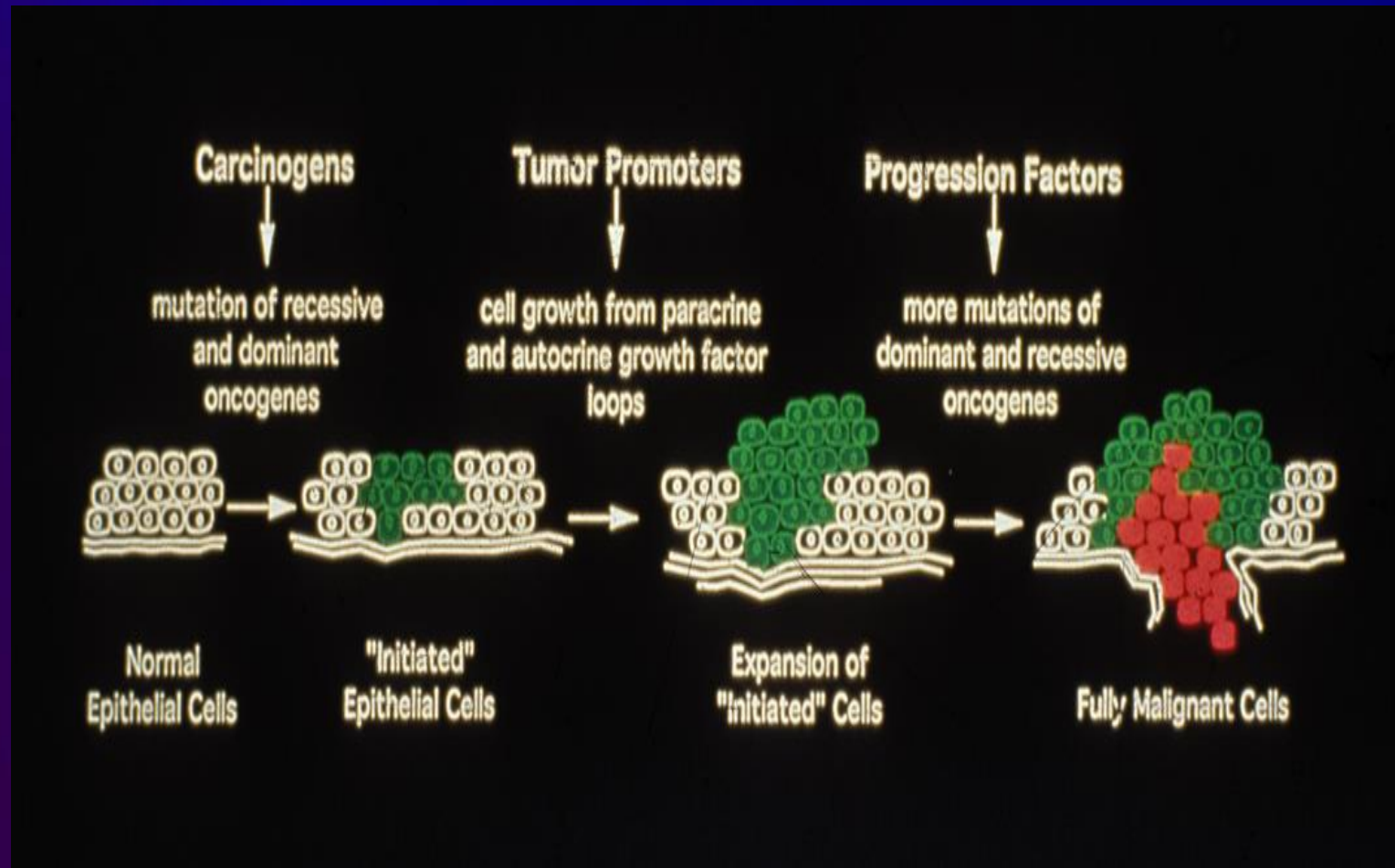
# Adenocarcinoma

- Chronic exposure to tobacco leads to malignant tumors such as adenocarcinoma.



# Tumor formation

- Growth factors promote carcinogenesis.
- Progression factors lead to malignant tumors.



# Tumor growth

## **Tumors**

- The primary cancer can undergo metastasis to distant organs.

**Carcinoma**



**Angiogenesis**



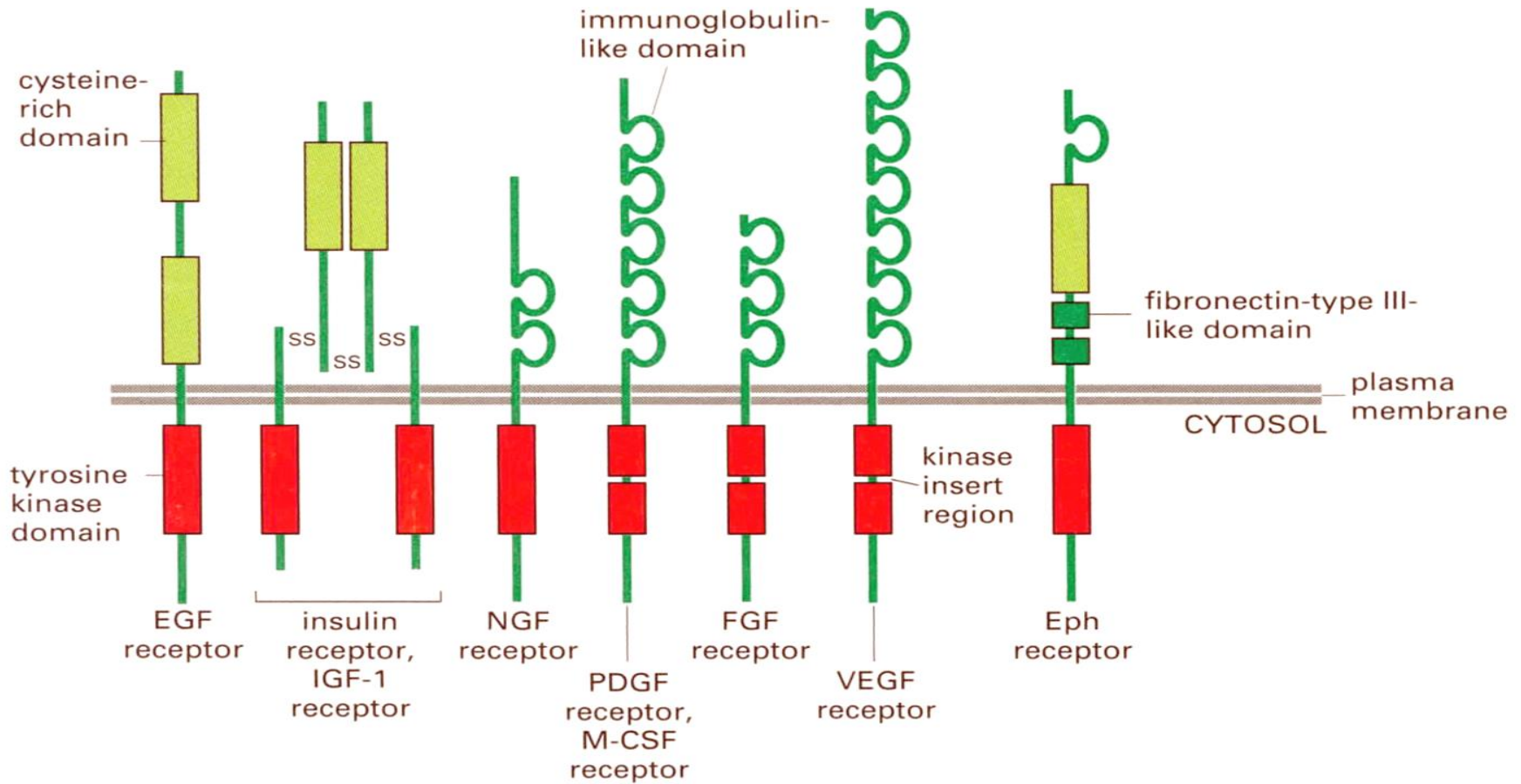
**Migration, Invasion and Metastasis.**

# Genetic abnormalities in lung cancer include:

- Mutation of tumor suppressor genes such as p53
- Silencing of tumor suppressor genes such as p16, Rb
- Amplification of oncogenes such as c-myc, cyclin D1, erbB-2
- Mutation of oncogenes such as K-ras, EGFR

# Tyrosine kinase receptors.

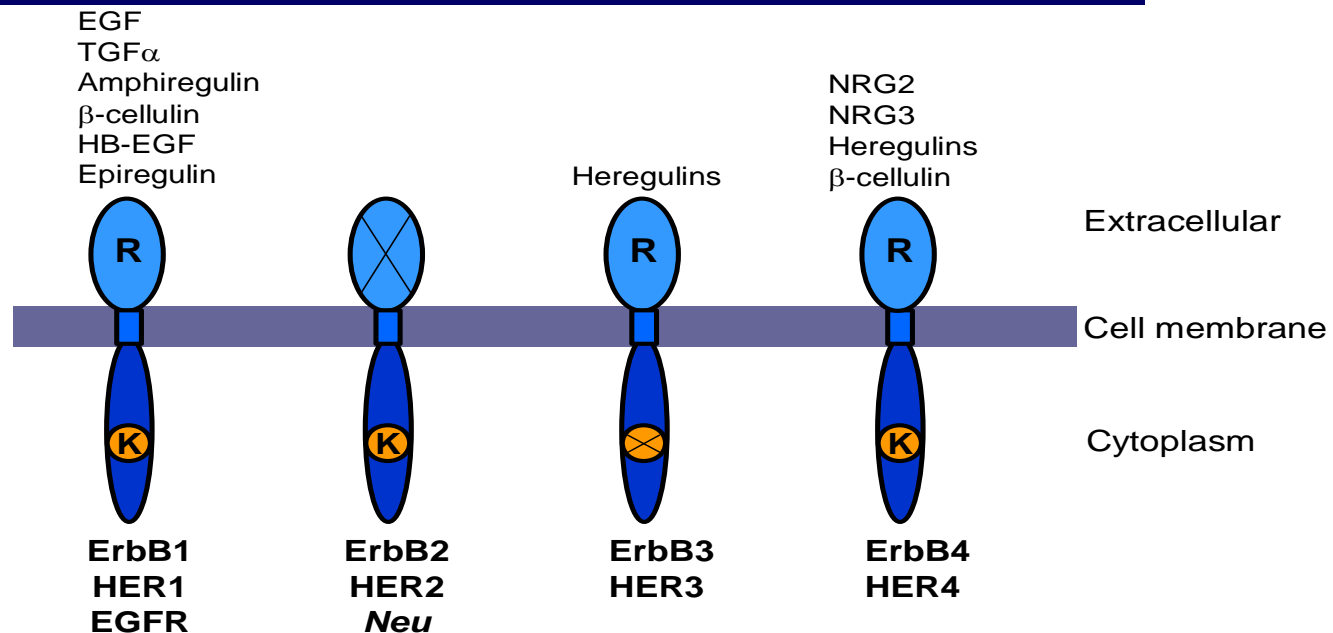
*Molecular Biology of the Cell, Alberts et al.,  
2001.*





# Tyrosine kinase receptors and ligands

## ErbB family of receptor tyrosine kinases (RTKs) and ligands



# **The EGFR is an 1186 amino acid integral membrane protein.**

- **The 621 amino acid extracellular domain binds EGF with high affinity. Domains I and III form the EGF binding site whereas domains II and IV are enriched in cysteine amino acids.**
- **The 24 amino acid transmembrane domain anchors the receptor into the membrane and transduces signaling.**
- **The 541 amino acid intracellular domain contains tyrosine kinase activity.**
- **Lys721 binds ATP and Tyr amino acids are subsequently phosphorylated.**
- **Tyr1068, 1086, 1148, 1174 are phosphorylated**

# EGF, TGF $\alpha$ and mAb 108 bind with high affinity to lung cancer cells.

Agent	IC <sub>50</sub> , ug/ml
EGF	.03
TGF $\alpha$	.8
TGF $\alpha$ -PE38	.4
mAb 108	3
IgG	>10

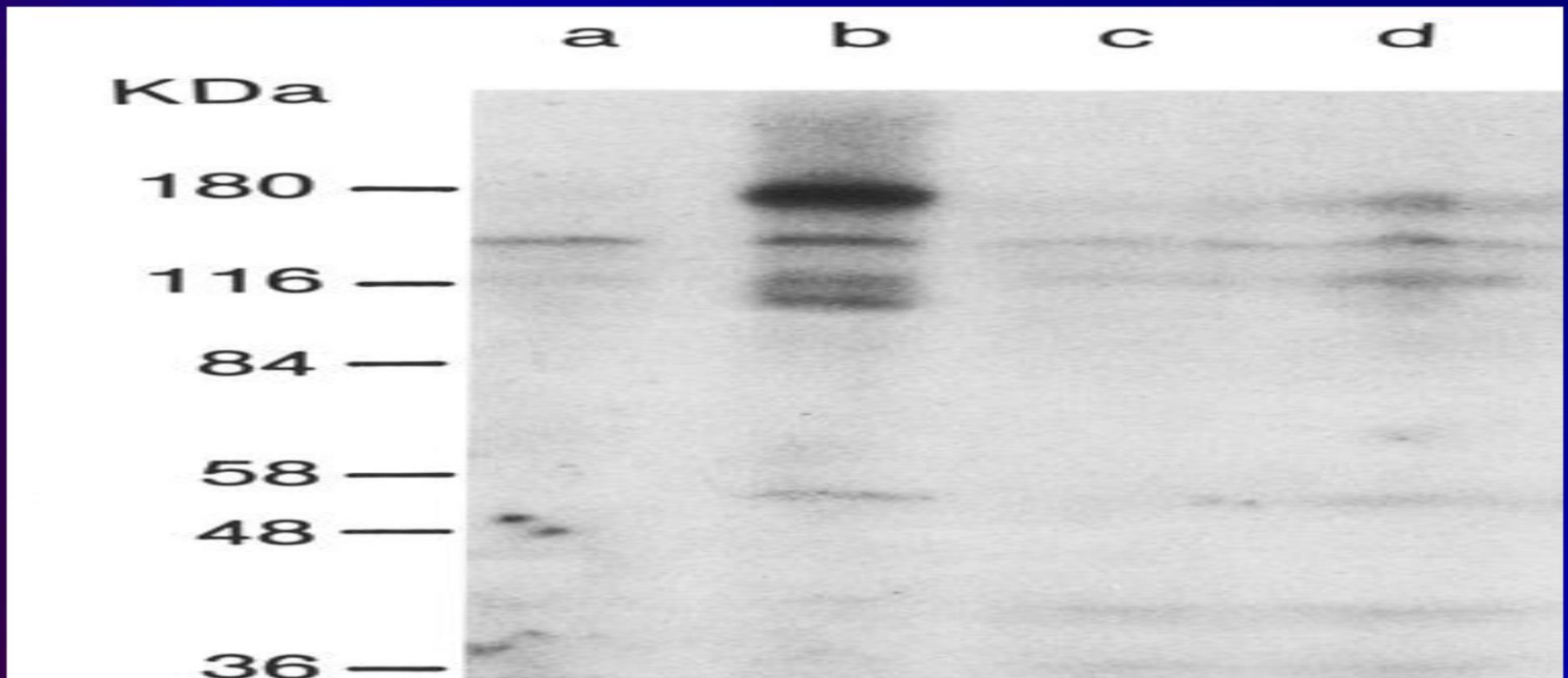
The IC<sub>50</sub> to inhibit <sup>125</sup>I-EGF specific binding to NCI-H157 cells was determined.

*Draoui et al., Life Sci. 1994; 35:352.*



# EGF tyrosine phosphorylation

**EGF causes tyrosine phosphorylation of the EGFR, PLC $\gamma$ , and PI-3-K.**

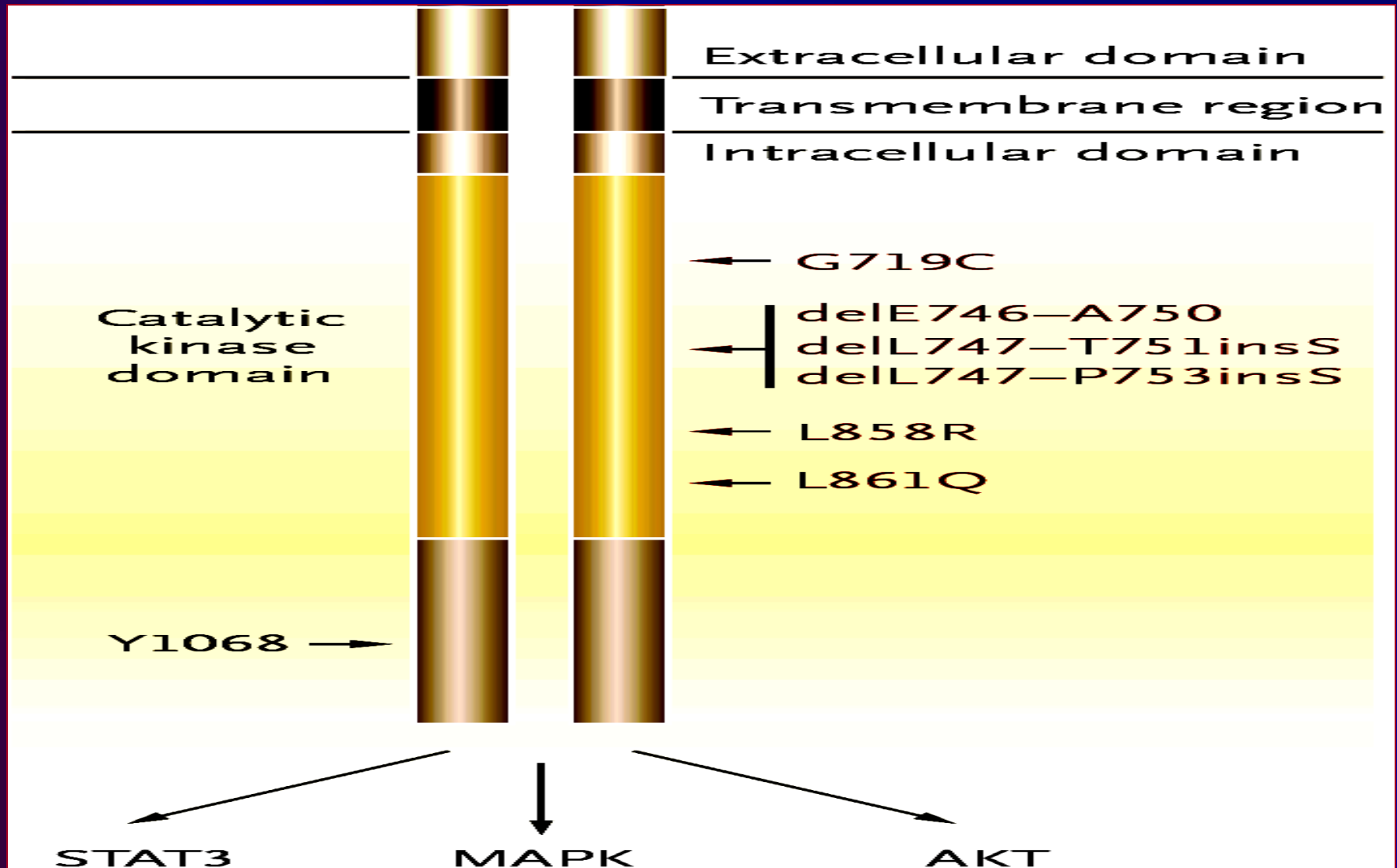


**Tyrosine kinase receptors are mutated in several diseases leading to increased cancer proliferation.**

- **EGFR mutations occur in the activation loop, especially L858R and G719C.**
- **Tyrosine kinase inhibitors (gefitinib and erlotinib) have been developed for the mutated EGFR.**

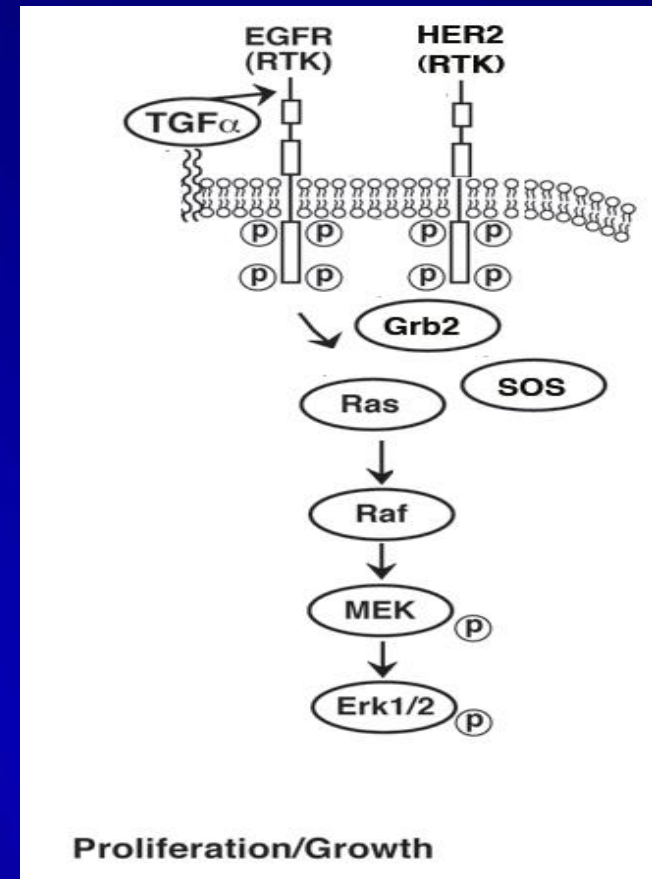
***Paez et al., Science 304:1497 (2004)***

# EGFR mutations



# RAS, RAF, MEK and ERK

- Receptor tyrosine kinases (RTK) stimulate proliferation Through the RAS, RAF, MEK and ERK pathway
- In NSCLC, K-RAS is Mutated in approximately 30% of the patients.



# RAS

- **Mutated RAS has reduced GTPase activity resulting in an abundance of biologically active RAS-GTP.**
- **Most of the RAS mutations are G-to-T transversions in codon 12.**
- **The FDA recently approved sotorasib (AMG510) for treatment of lung cancer patients with the KRAS G12C mutation.**

# RAF

- **RAF is a serine threonine kinase which activates MEK. B-RAF-V600E mutations occur in approximately 60% of melanoma patients leading to an active kinase.**
- **PLX4032 is a kinase inhibitor which has an 81% response rate in patients with metastatic melanoma.**
- **RAS and B-RAF are driver mutations in several types of cancer.**

# MEK

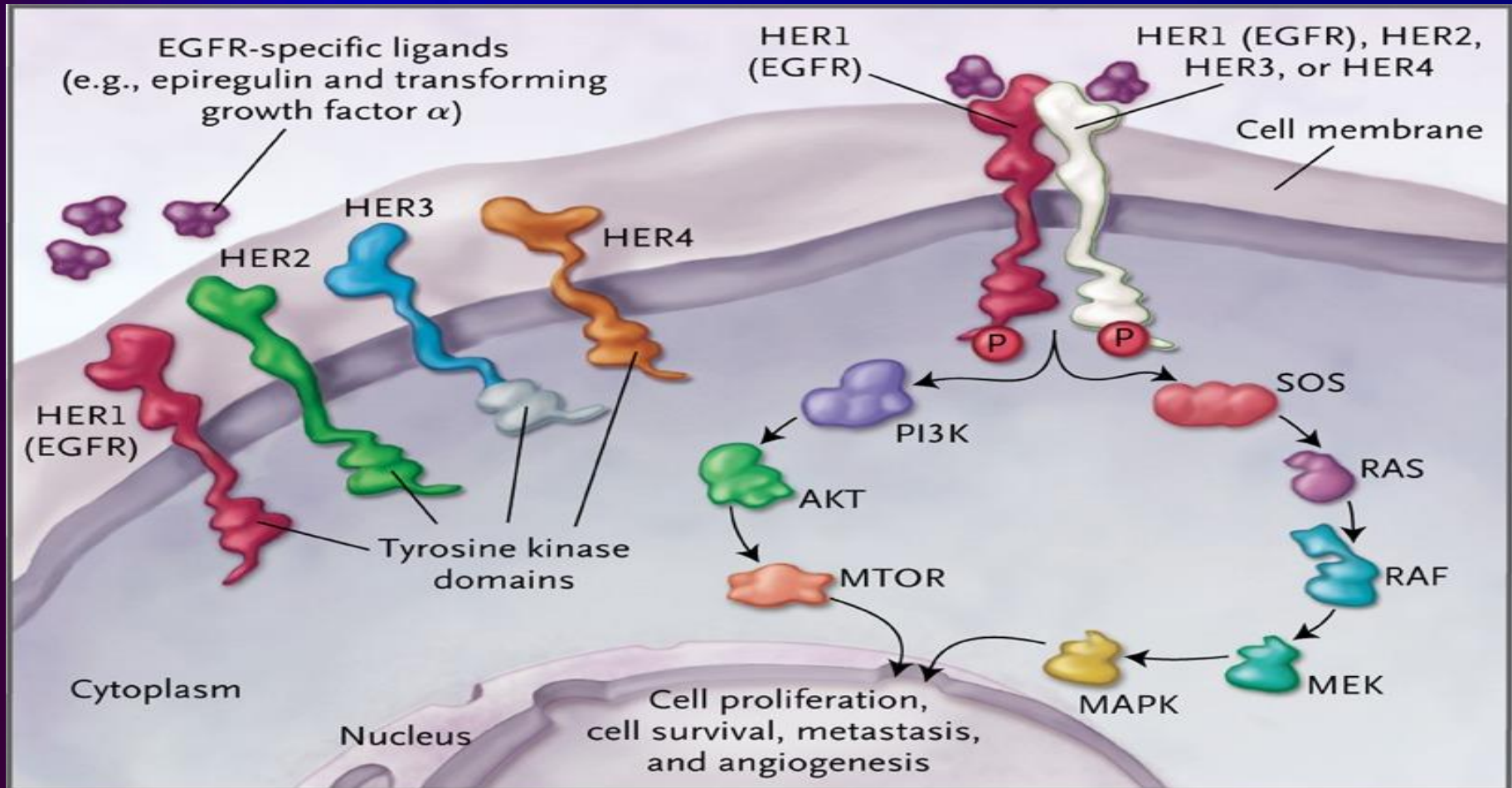
- **RAF phosphorylates mitogen activated protein kinase kinase (MEK) increasing its activity.**
- **MEK1 and MEK2 are inhibited by trametinib**
- **Dabrafenib and trametinib are used in B-RAF mutated patients.**

# ERK

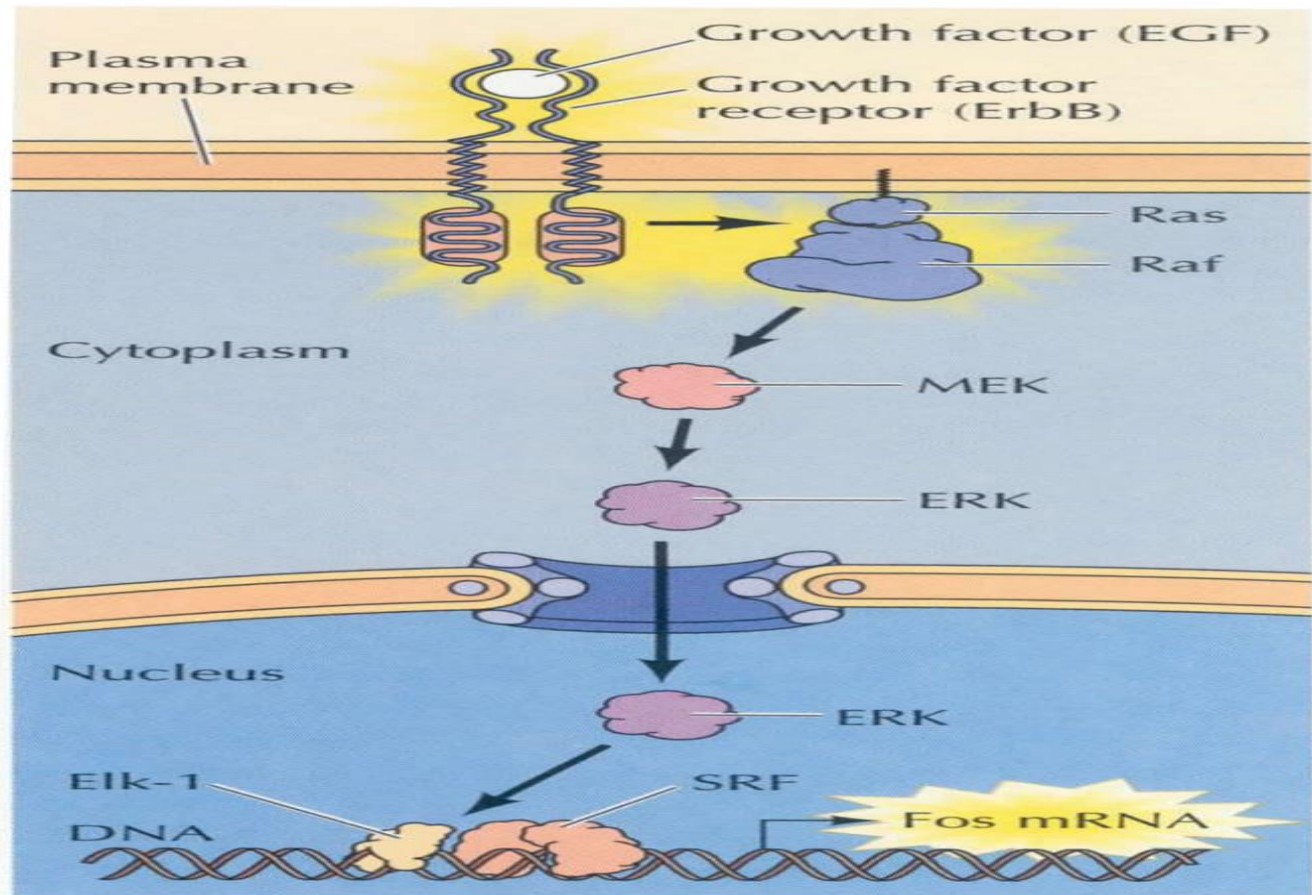
- **MEK1/MEK2 regulates the phosphorylation of extracellular signal-regulated kinases (ERK) 1 and 2.**
- **Phosphorylated ERK goes to the nucleus where it regulates expression of transcription factors such as fos, jun or myc.**



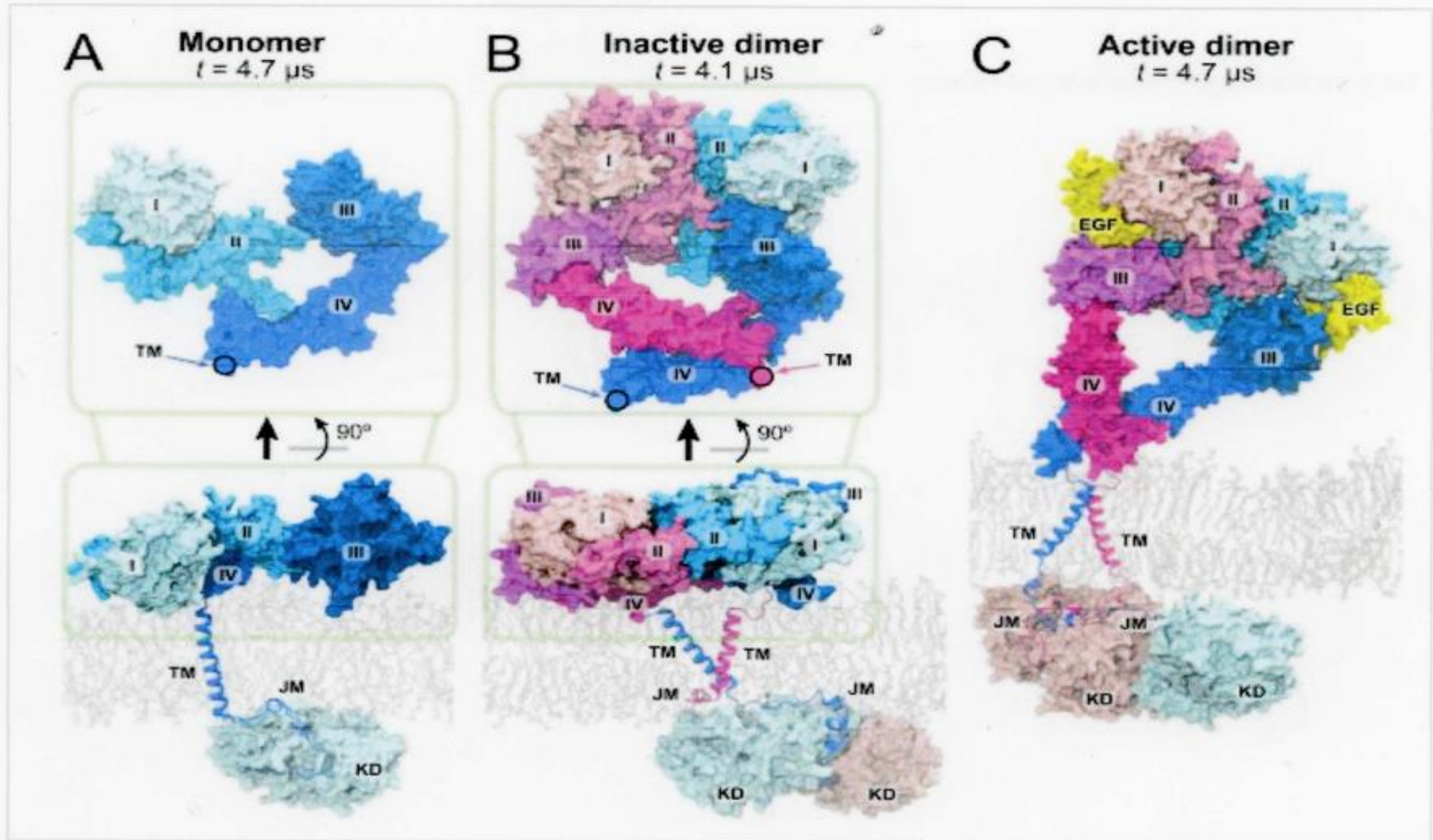
# ErbB RTKs and signal transduction



# The EGFR stimulates cancer cell growth. *Molecular Biology of the cell; Alberts et al., 2001.*

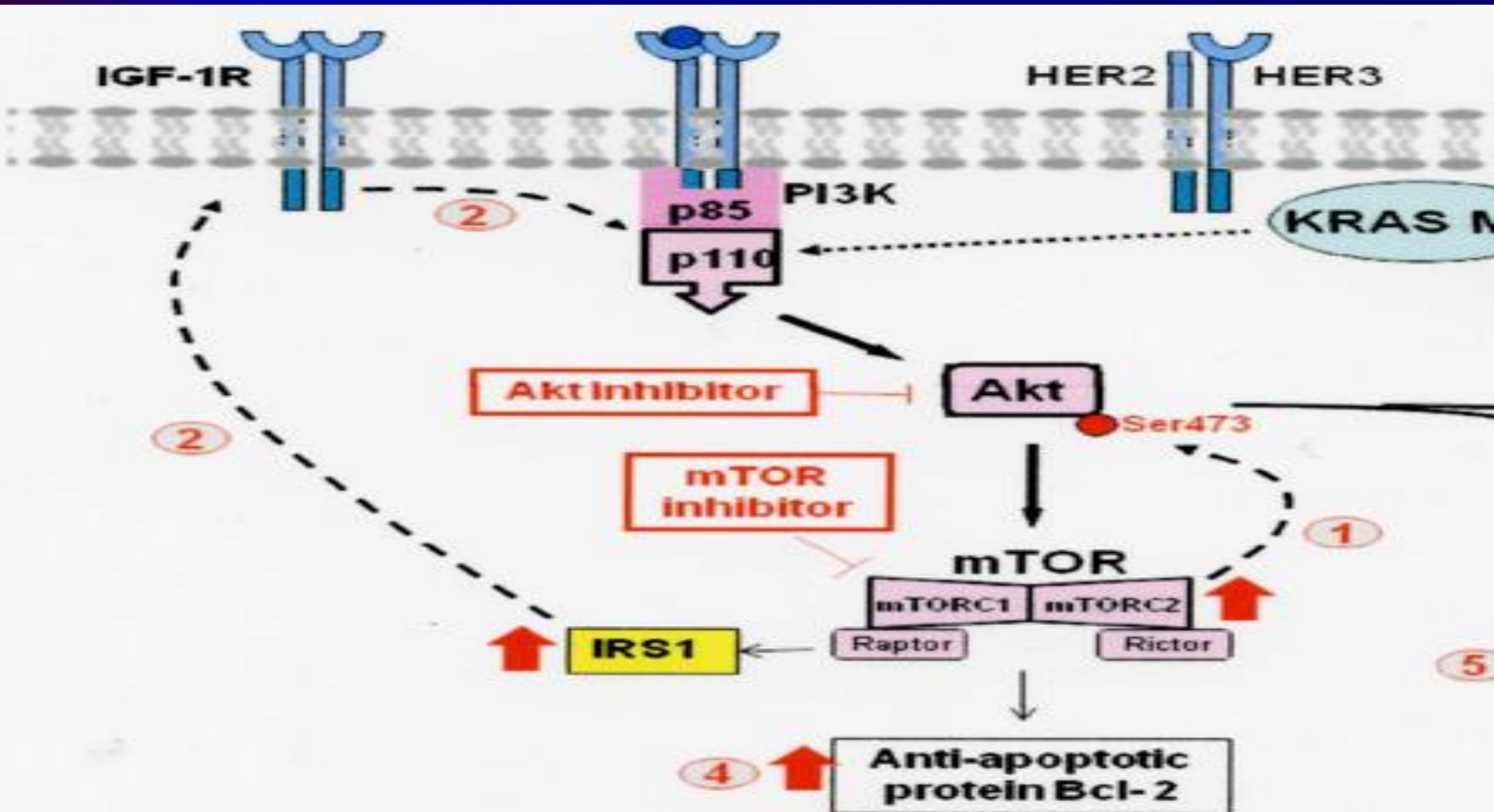


# EGFR dimerization





# PI3K, Akt, mTOR pathways stimulate cellular survival.



# PI3K

- The phosphatidylinositol 3 kinase (PI3K) pathway promotes cancer cell survival.
- The catalytic 100 kDa subunit metabolizes  $\text{PIP}_2$  to  $\text{PIP}_3$
- PI3K is mutated in breast (25%), brain (27%), colon (30%) and stomach (25%) at E542, E545 or H1047 resulting in a gain of enzymatic activity.

# PTEN

- PI3K mutations involve chromosome 10q, which contains phosphatase and tensin homolog (PTEN).
- PTEN metabolizes  $\text{PIP}_3$  to  $\text{PIP}_2$  leading to inhibition of AKT signaling.
- PTEN is mutated in approximately 13% of breast cancer patients but loss of heterozygosity is more common.

# Akt

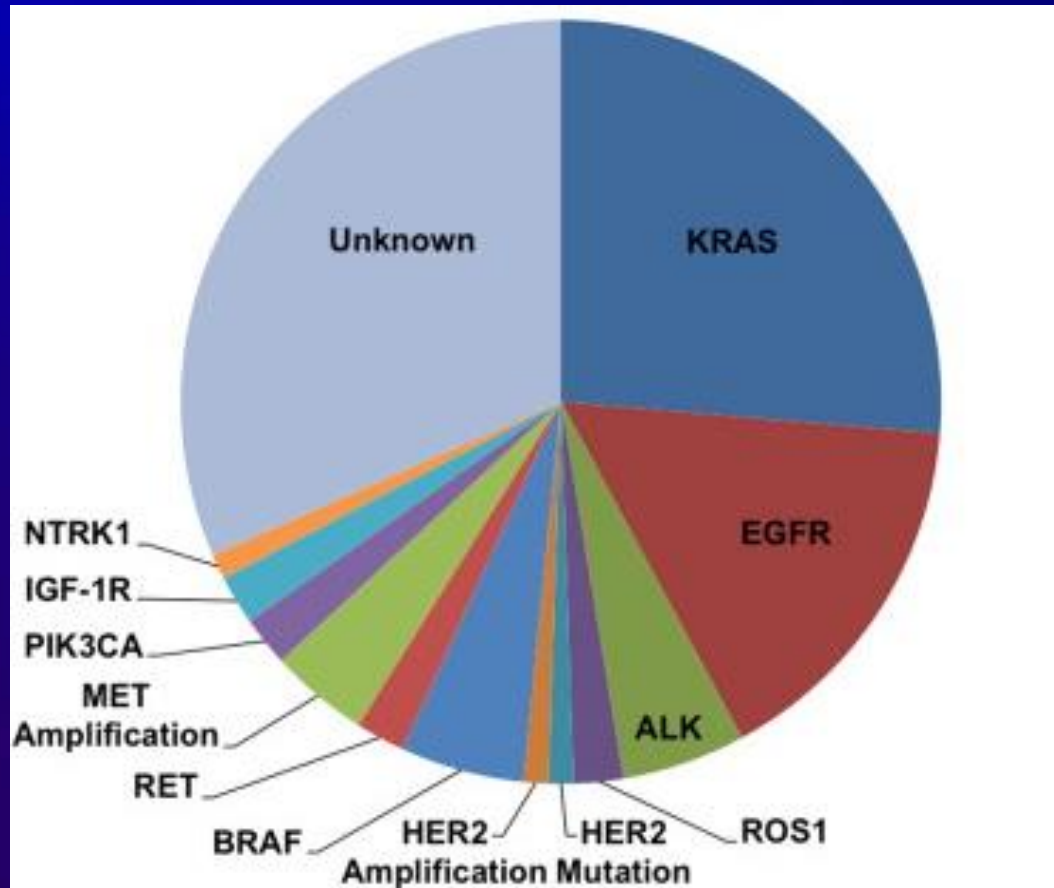
- **AKT or protein kinase B prevents apoptosis of cells.**
- **AKT is a serine/threonine kinase which is phosphorylated at Ser473 increasing phosphorylation of mTOR.**
- **AKT promotes cellular survival by phosphorylating BAD and caspase-9 preventing apoptosis of cancer cells.**
- **AKT is mutated in breast cancer (5%), colorectal cancer (6%) and ovarian cancer 2%.**

# mTOR

- Mammalian target of rapamycin (mTOR) or FRAP1 is a serine/threonine kinase.
- mTOR activation enhances phosphorylation of p70S6 kinase and 4E-BP1 increasing protein translation and cellular proliferation.
- mTOR activation decreased autophagy, a lysosome-dependent degradation pathway.



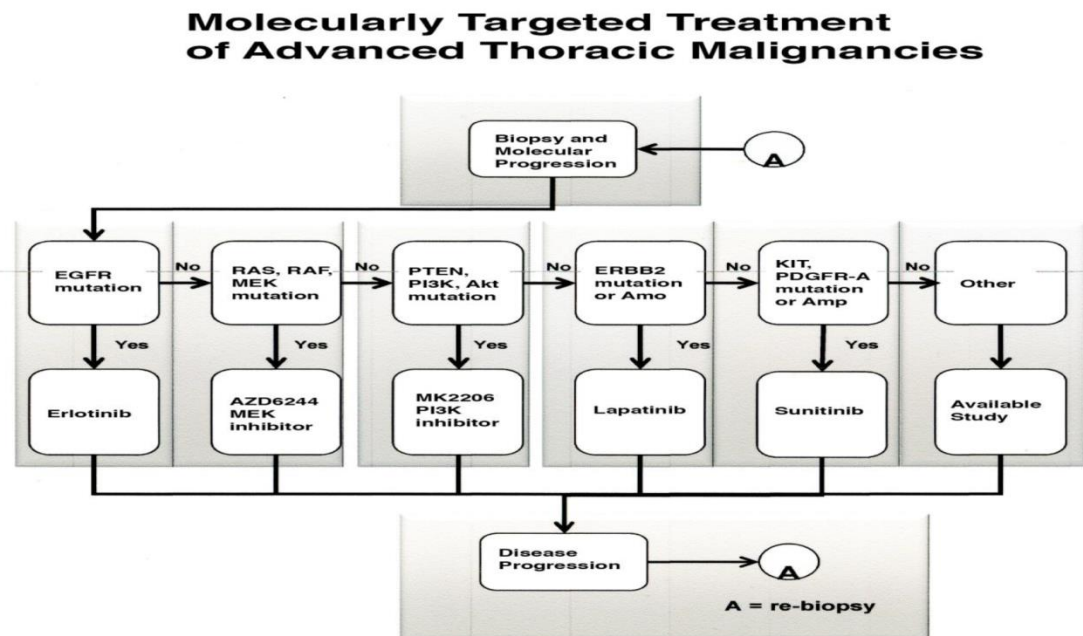
# Personalizing Therapy for NSCLC Genetic Abnormalities in Lung Adenocarcinoma



# Molecular medicine

## Molecularly Targeted Treatment of Advanced Thoracic Malignancies

**Patient Selection:  
Molecular analysis  
of lung cancer  
malignancies**

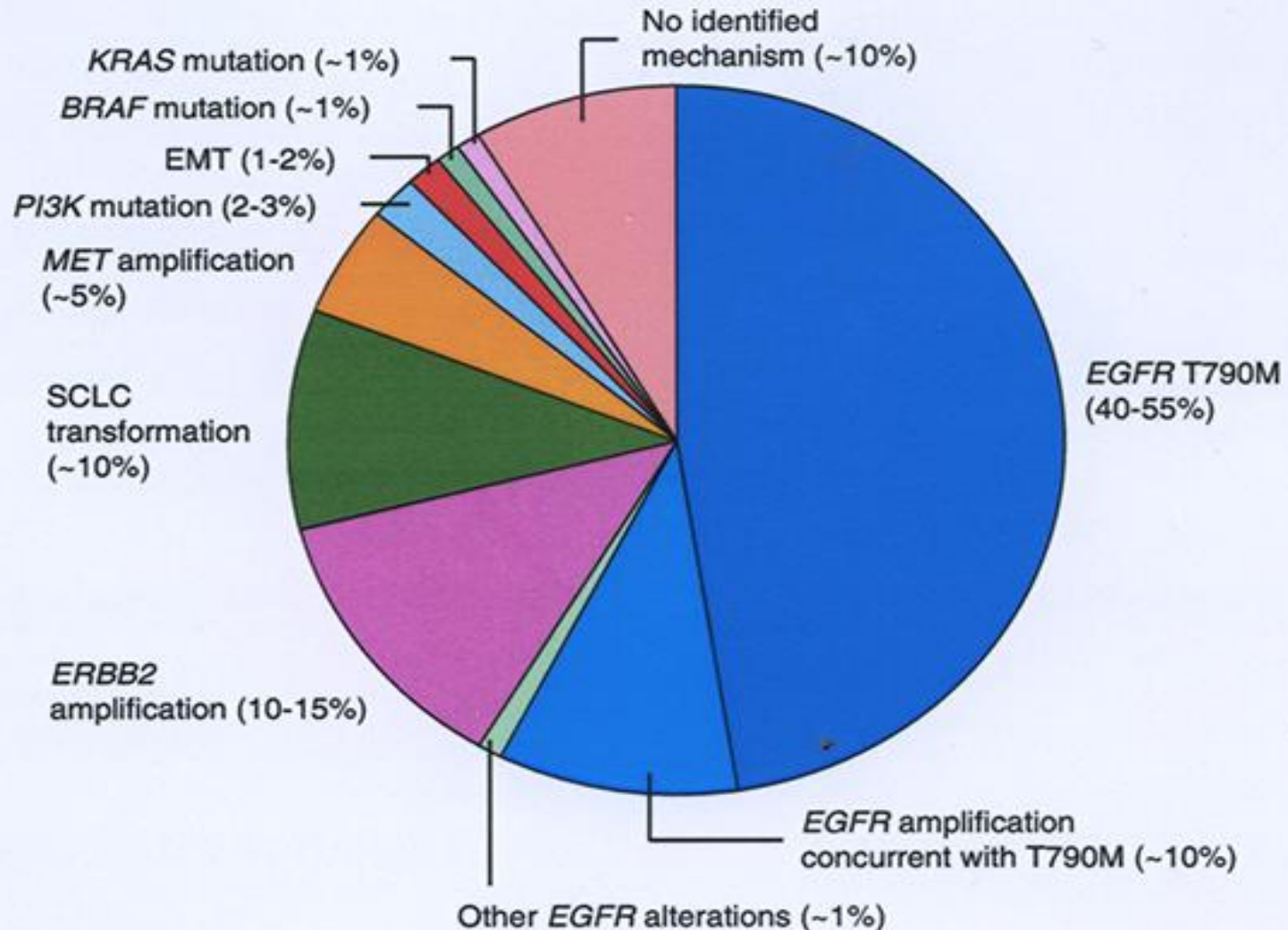


# **Erlotinib/gefitinib resistance**

- **NSCLC patients with L858R EGFR mutations develop resistance to erlotinib/gefitinib after 1 year due to a secondary mutations.**
- **Aproximately 50% of the patients develop T790M mutations which can be treated with Osimertinib.**

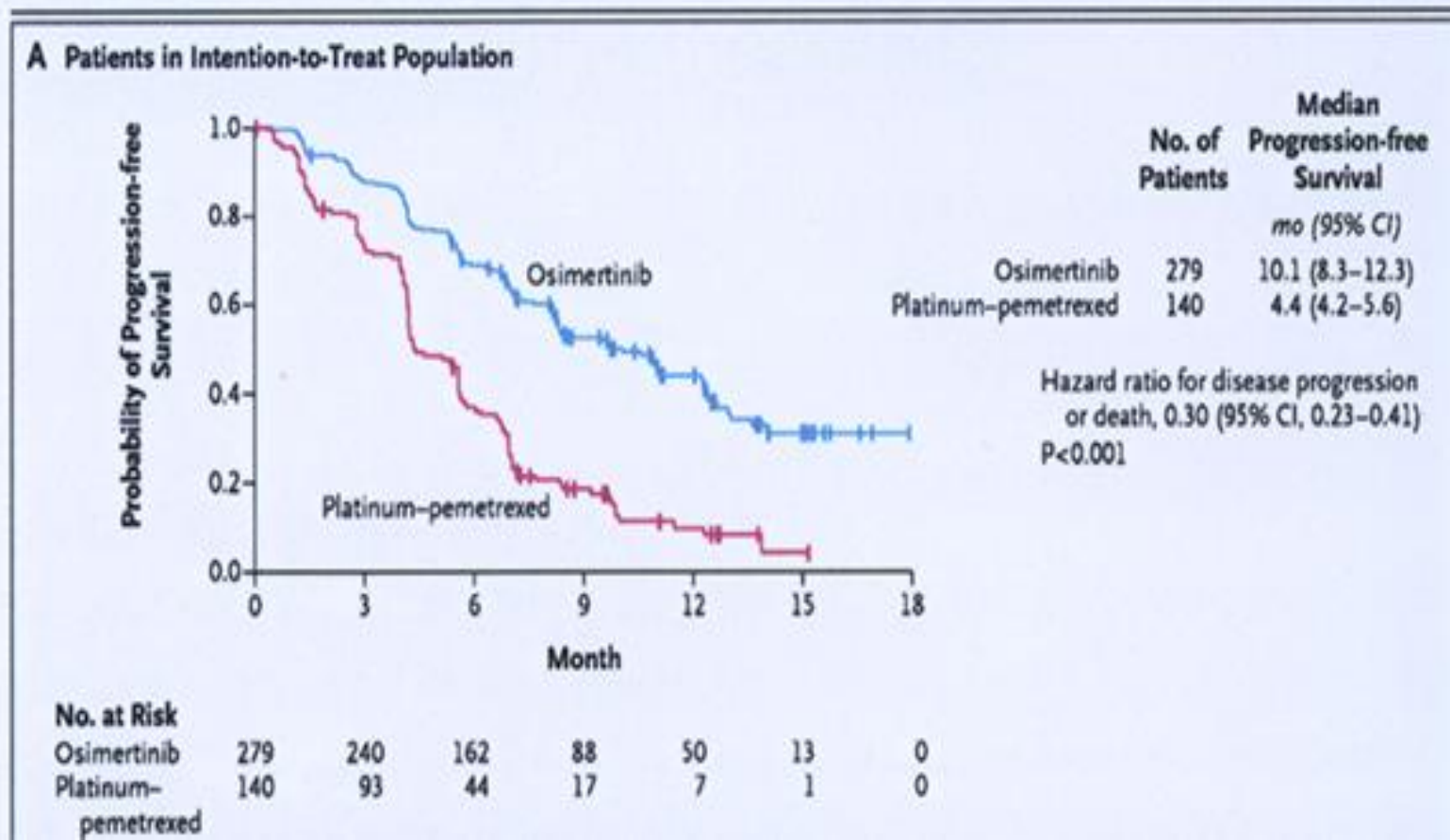
# Secondary mutations in NSCLC

*Westover et al., Ann Oncol. 2018*



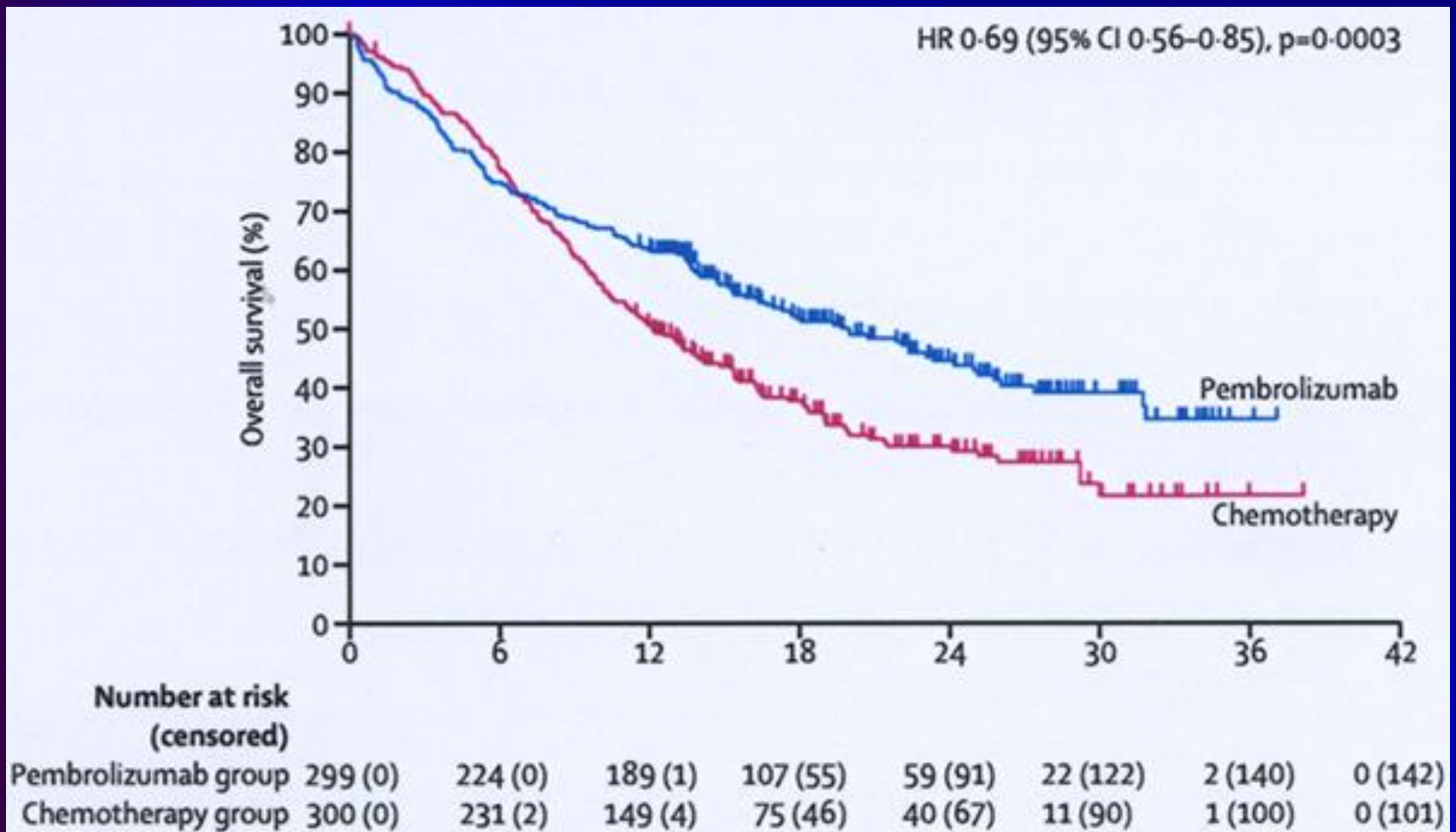
# Osimertinib improves overall survival in EGFR T790M patients

*Mok et al., N Engl. J Med 2017*





# Immune checkpoint inhibitors such as pembrolizumab increase overall survival in patients with PD-L1



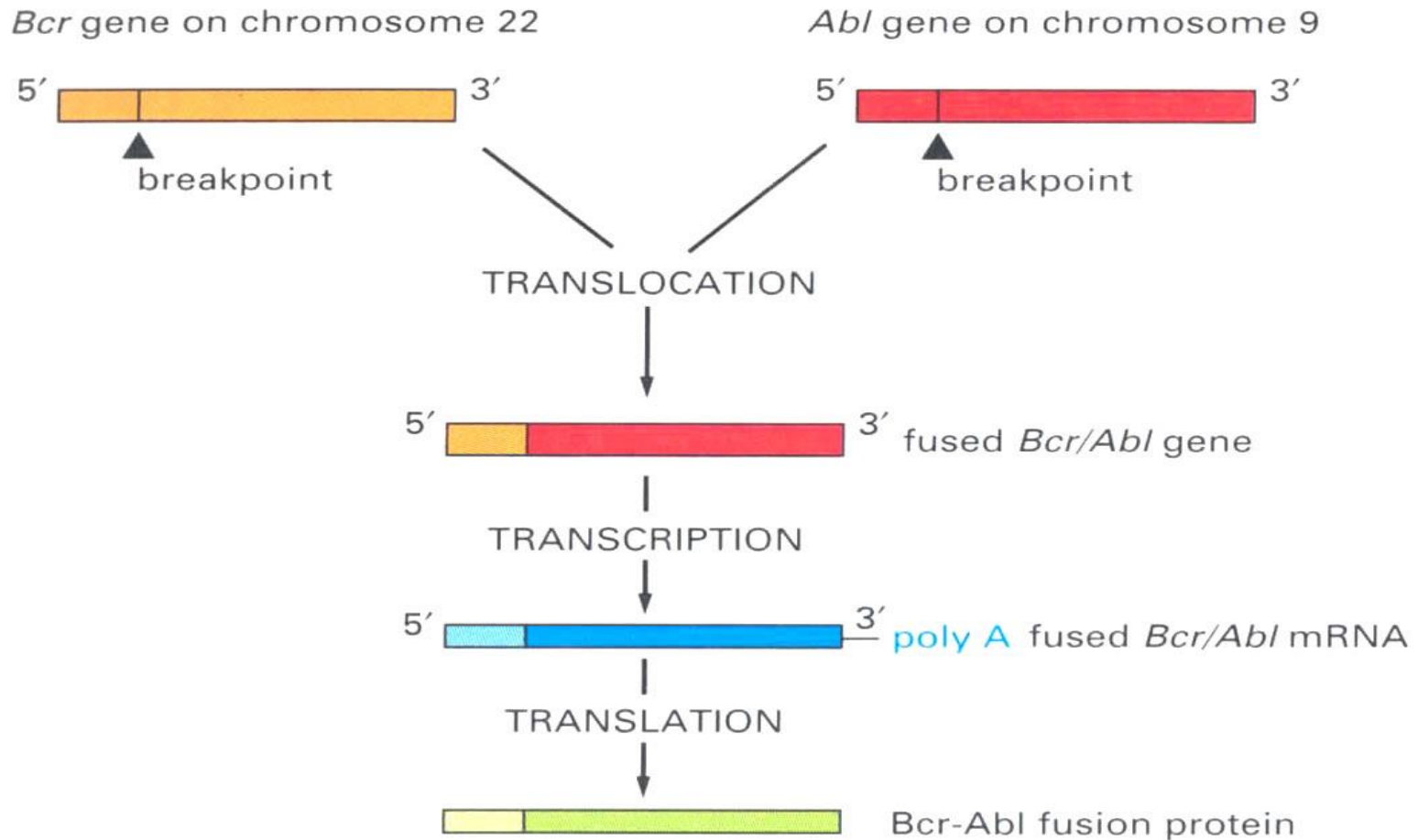
# CML patients

**CML patients have a genetic abnormality on chromosome 22 (Philadelphia chromosome).**

- Segments of chromosome 9 and 22 are fused resulting in the bcr-abl gene.
- The resulting tyrosine kinase is constitutively active.
- Bcr-abl tyrosine kinase activity is inhibited by Gleevec.

# Translocation of Bcr/Abl.

- Chromosome 22 translocates with chromosome 9. Molecular Biology of the Cell; Alberts et al., 2001.

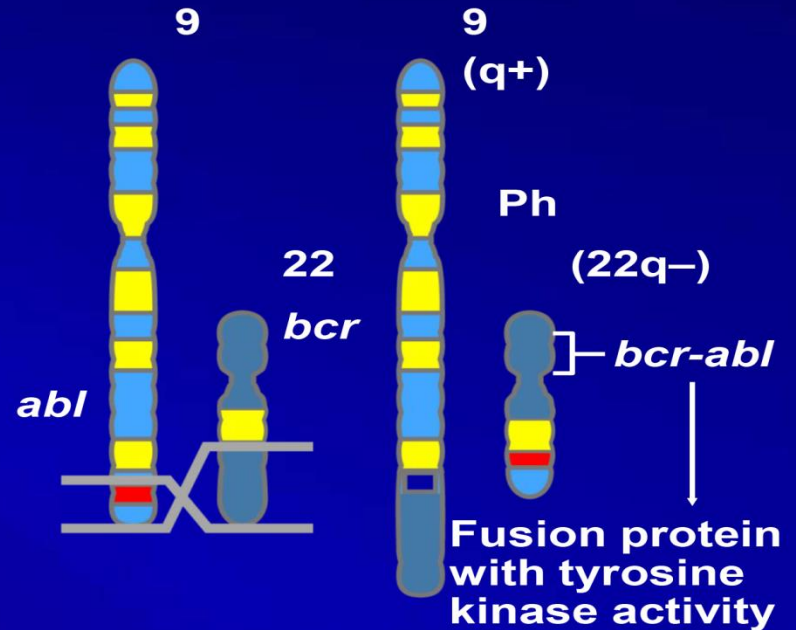
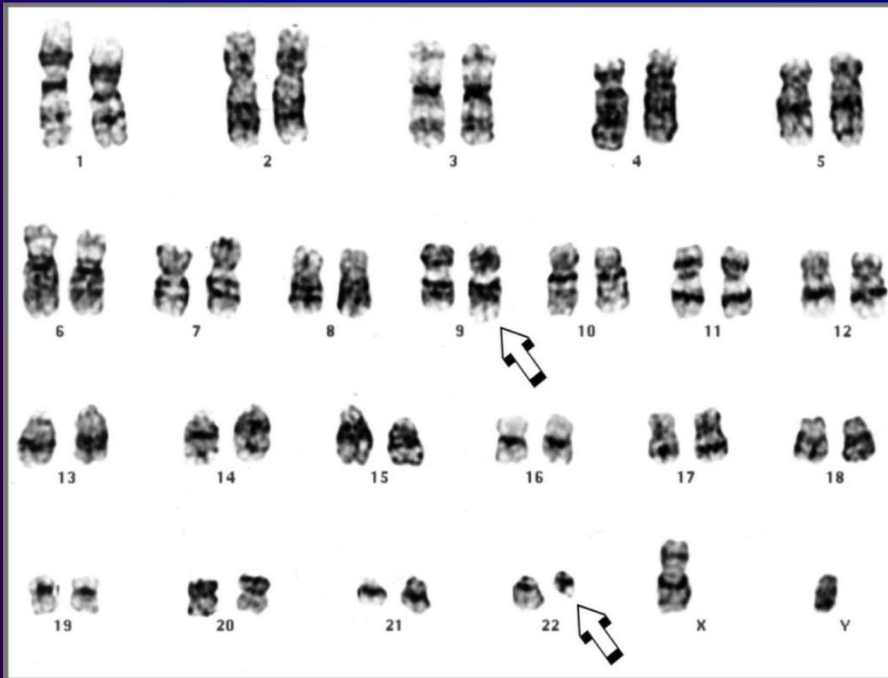




# Bcr-Abl

## Translocation of *Bcr-Abl* Genes

● Translocated chromosome 9 appears larger and translocated chromosome 22 appears smaller: “Freebies for Teachers”; D. Kerrigan.



**In a Phase I Clinical Trial,  
Gleevec™ was effective orally at  
a daily dose of 300 mg or greater.**

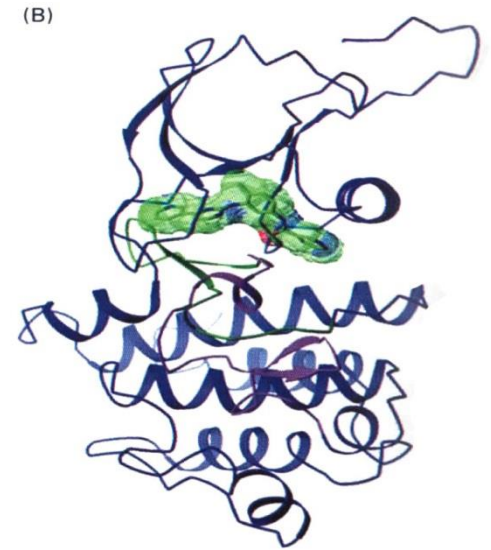
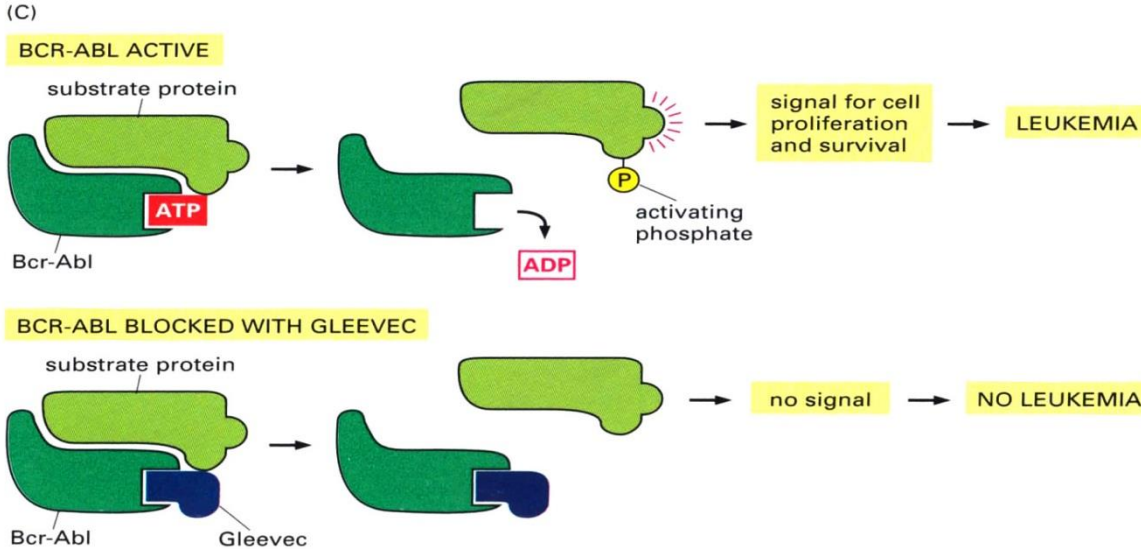
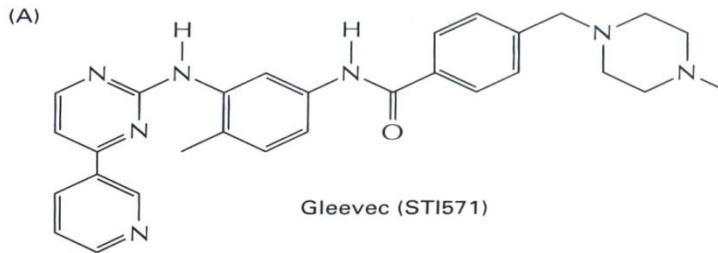
- **Dose limiting toxicities included  
nausea, vomiting, edema and rash.  
(Sawyers and Druker. Cancer J. Sci.  
Am. 1999;5:63).**

# **In a Phase II Clinical Trial, Gleevec™ restored normal blood counts in 53 out of 54 chemotherapy- resistant CML patients.**

- After a year on Gleevec, 51 of these patients were still doing well. (Druker et al. N. Engl. J. Med. 2001; 344: 1038.).
- Over a 5 year period, 89% of the patients treated with Gleevec had progression-free survival (O'Hare et al., Clin. Cancer Res. 2011; 17: 212).

# Gleevec mechanism of action

- Gleevec blocks the ATP binding site. Molecular biology of the cell; Alberts et al., 2001.



# **PRACTICAL STEPS TO PREVENT CANCER**

- **Check your house for radon.**
- **Check your house for asbestos.**
- **Take precautions at your workplace.**
- **Check your community water system.**
- **Avoid breathing polluted air.**
- **Protect your skin.**
- **Don't breathe smoke.**
- **Exercise daily.**

# Cancer prevention

## **PRACTICAL STEPS TO PREVENT CANCER (continued)**

- **Avoid pesticides.**
- **Eat fruits and vegetables.**
- **Reduce red-meat consumption.**
- **Eat fish.**
- **Minimize fried foods.**
- **Drink alcohol in moderation.**
- **Avoid unnecessary x-rays.**
- **Reduce infections.**

# • REFERENCES

●Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist, RS. Lung cancer. Lancet, 2021. PMID 34273294.

Westover D, Zugazagoitia, J, Cho BC,

●Lovely CM, Paz-Ares L. Mechanisms of acquired resistance to first-and second-generation EGFR tyrosine kinase inhibitors. Ann Oncology 2018; 29: 110-119.